REMARKS/ARGUMENTS

By Office action dated May 18, 2004, prosecution was reopened. To avoid abandonment of the application, Applicant was required to either file a reply to the non-final Office action or to request reinstatement of the appeal. Applicants have chosen to respond to the Office action mailed May 18, 2004. Claims 1-8, and 22-30 are pending and under examination. Claims 1-3, 5, 6, 8, 22-24 and 29-30 are currently amended in order to further Applicants business interests and the prosecution of the present application, not for reasons of patentability. The amendments to the claims are adequately supported by the specification, for example, at pages 12-15 and 19-24.

In conformity with proposed U.S. Patent and Trademark Office rules set forth in OG Notice 25 February 2003, Applicants have included a complete detailed listing of the claims under the section, "Amendments to the Claims." The detailed listing presents all claims that are, or were, in the application. The current amendments to the claims are expressed in the listing. The requirement to provide two versions of a replacement paragraph, section, or claim (a clean version and a marked up version), as set forth in current 37 C.F.R. § 1.121, is waived per the cited OG Notice.

For the sake of clarity, the rejections and objections of the presently outstanding Office Action are set forth below, in the order in which they were presented and are herein addressed:

1. Claims 1-8, and 22-30 are rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement.
2. Claim 8 is rejected under 35 U.S.C. § 112 second paragraph as being indefinite.

1. Claims 1-8, and 22-30 are enabled.

Claims 1-8, and 22-30 are allegedly rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement. Specifically the claims are rejected for lack of enablement as

allegedly (a) having unpredictability and requiring undue experimentation; (b) the declaration of Dr. Judith Campisi is not persuasive as objective evidence; and (c) not providing in vivo animal model data and lack of examples.

Applicants herein address the rejections in the order as laid out in the Office Action. Applicants traverse each aspect of the rejection and show (a) that the claims are predictable and do not require undue experimentation; (b) that the declaration of Dr. Judith Campisi is persuasive evidence that in vitro cell line models are accepted as predictive and correlative of tumorigenicity in vivo; and (c) that the claims do not require objective evidence for enablement.

A. That the claims are predictable and do not require undue experimentation.

The Office Action alleges that "the level of skill in the art in cancer diagnosis using a newly discovered biomarker such as α-dystroglycan in blood or serum is low and cancer diagnosis art is unpredictable," thereby requiring that Applicants supply a working in vivo example. Applicants believe that this argument is meant to imply that because of the allegedly low level of skill of those in the art and the alleged unpredictability of cancer diagnosis, that the Office takes the position that the practice of the claimed invention is unpredictable, requiring undue experimentation unless an in vivo working example is provided. These rejections are traversed.

Applicants disagree that the level of skill in the art in cancer diagnosis is low. As Applicants stated in the previous response mailed May 2, 2003, the leading case on enablement, *In re Wands*, 8 UPSQ2d 1400 (1988), discussed at MPEP 2164.01, involved an immunoassay and found that the level of skill in this art was very high.

Furthermore, Applicants take issue with the assertion that cancer diagnosis art is unpredictable. The Office Action states that '[i]n order to use the full scope of the claimed invention, one skilled in the art has to screen a large quantity of clinical samples to

determine whether detecting molecular weight of 120-130kD or 60 kD fragment of α-dystroglycan in blood and/or serum, is correlated with a higher tumorigenicity..." (Office action, page 4, paragraph 2). Applicants disagree with the Office action's conclusion yet do agree with the statement on page 13 of the Office action, "[t]he Office does not require applicant to submit data for efficacy in human clinical trials." Since the Office does not require the submission of data for efficacy in human clinical trials, then such efficacy and data from screening a large quantity of clinical samples is improperly required to find for enablement of the full scope of the claimed invention. The application provides laboratory and prophetic data as support for the claims. All of this has been corroborated by the Declaration of Dr. Judith Campisi, and others in the art.

The Office Action cites as support that cancer diagnosis is unpredictable, Wirth et al. of record (*Eur Urol* 1993; 24 Suppl 2:6-12) and Tockman et al. of record (*Cancer Res.*, 1992, 52:2711s-2718s). Wirth et al, describe PSA. Although PSA is known to be limited in its specificity between benign hyperplasia and prostate cancer, however, this has not prevented its commercial application as an extremely valuable prostate tumor marker used by countless clinicians. Applicants assert that Tockman et al. merely teach that prior to successful broad commercial application of newly described early stage markers, certain criteria should be met. Applicants respectfully assert that the rejection overlooks the distinction between data required for broad commercial application and data required by the case law and the Office for application for a patent. Applicants further address the level of proof and evidence sufficient for an asserted utility *infra* in Section C which addresses the improperly-based requirement of in vivo objective evidence.

On pages 5-6 of the rejection, the Office again cites Tockman et al, for the premise that "prior to the successful application of newly described markers, the markers must be validated against acknowledged disease end points and the marker predictive value must be confirmed in prospective population trials (p. 2716s, col.2)." (Office action, p.6). Again,

Applicants respectfully argue that Tockman et al does not apply here, and requiring Applicants to submit data for the efficacy in human clinical trials or confirmation in population trials, is improper and not required.

Applicants take issue with the Office action's mischaracterization of Applicant's claimed invention on page 7 of the rejection. The inventors of the claimed invention are not baldly claiming that their assertions alone should be accepted as enabling disclosure, as the Office so implies. (Ibid). Applicants describe in the specification how to make and use the claimed invention, for example on pages 5-8, 13-14, and17-20. Working examples are provided showing that α-dystroglycan is proteolysed and shed from the cell surface of tumor cells (Examples 1 and 2), that over-expression of dystrolgycan restores normal dystroglycan function of some tumor cell lines (Example 3), that addition of a metalloprotease inhibitor restores normal dystroglycan function of several tumor cell lines (Example 5), and that assays can be performed to detect the α-dystroglycan cleavage event (Example 4). Furthermore, as set forth in the arguments below, such evidence is sufficient to enable the skilled artisan to make and use the claimed invention. Moreover, the Declaration of Dr. J. Campisi, of record, has been submitted, which confirms the enablement and utility of the present invention.

Applicants respectfully disagree that a working in vivo example is required (see Section C which addresses the improper requirement of in vivo objective evidence). The Office alleges that there is no working example of "a method of measuring potential tumorigenicity of cells by detecting presence of α-dystroglycan on cell surface, more specially relative decrease of α-dystroglycan as compared to β-dystroglycan indicates a higher potential tumorigenicity." (Office Action, page 4). Applicants disagree and assert that there is working example and point to support in Example 2 and further support on pages 3, 9-10, and 19-20. See also the Campisi Declaration, of record.

The Office action implies that the claimed invention is unpredictable unless Applicant can present evidence that α-dystroglycan is not shed by normal tissues. Applicants point to Exhibit E, Sgambato A, et al., Dystroglycan expression is frequently reduced in human breast and colon cancers and is associated with tumor progression, *Am J Pathol.* 2003 Mar;162(3):849-60, which is attached. Applicants bring to the attention of the Examiner, Figure 5A and C of Sgambato et al. specifically, which describe and show immunohistochemical staining of normal breast tissues and tissues having high grade carcinoma. In the Figures, α-dystroglycan was distinctly detected in normal tissues but lost in tumorigenic tissues. This reinforces that there is differential detection between normal and diseased tissues. However, the concept here is <u>not</u> that there is no baseline shedding. Rather, it is that Applicants have found that shedding of α-dystroglycan increases in diseased states.

Applicants assert that the predictability of Applicants' claimed invention is high because <u>others in the art have confirmed the correlation</u> of "a method of measuring potential tumorigenicity of cells by detecting presence of α-dystroglycan on cell surface, more specially relative decrease of α-dystroglycan as compared to β-dystroglycan indicates a higher potential tumorigenicity." (Office Action, page 4). In support of this, Applicants herewith submit as Exhibits, references by others in the art that have shown that Applicant's in vitro working examples correlate with the claimed invention. The Exhibits include:

- **Exhibit A,** Sgambato A, et al., Increased Expression of Dystroglycan Inhibits the Growth and Tumorigenicity of Human Mammary Epithelial Cells, *Cancer Biol Ther.* 2004 Oct 2;3(10) [Epub ahead of print], which also confirms Applicant's claimed invention.

- **Exhibit B,** Jing J, et al., Aberrant expression, processing and degradation of dystroglycan in squamous cell carcinomas, *Eur J Cancer.* 2004 Sep;40(14):2143-51,

which describes the reduction or loss of α-dystroglyacan in all oral cancer samples and cell lines.

- **Exhibit C,** Brennan PA, et al., Dystroglycan complex in cancer, *Eur J Surg Oncol.* 2004 Aug;30(6):589-92, which is a review describing α-dystroglycan and its role in cancer.

- **Exhibit D,** Weir ML, et al., Dystroglycan: emerging roles in mammary gland function, J Mammary Gland Biol Neoplasia. 2003 Oct;8(4):409-19, a review co-authored by one of the inventors describing dystroglycan and its role in breast cancer.

- **Exhibit E,** Sgambato A, et al., Dystroglycan expression is frequently reduced in human breast and colon cancers and is associated with tumor progression, *Am J Pathol.* 2003 Mar;162(3):849-60, which describes and confirms in in vivo human primary prostate and breast cancers and cancer cell lines that α-dystroglycan levels are lost or reduced, that progressive loss of α-dystroglycan tracks advancing tumor progression stages, and that there is a significant correlation between loss of α-dystroglycan and overall patient survival rate.

To allege that undue experimentation would be required, the Office action also cites Henry et al., (Human Pathology, vol. 32, pages 791-795, 2001) as teaching that both in vivo prostate and breast tumors, β-dystroglycan detection is reduced. (Office action, page 8). The Office takes the position that one skilled in the art would be required to resort to undue experimentation to resolve the differences shown in Henry et al., and applicant's assertion that absence of or detecting a relative decreased amount of α-dystroglycan on cell surface as compared to β-dystroglycan is indicative of higher potential tumorigenicity. Applicants respectfully point out that Henry et al. do not address the reduction of expression of α-dystroglycan in breast or prostate cancers because, as the rejection concedes, Henry et al. only used an antibody specific for β-dystroglycan. (Human Pathology, vol. 32, page 791). Furthermore, the fact that this reference asserts that β-dystroglycan was undetectable in prostate adenocarcinoma has no bearing on Applicant's currently claimed invention. Over and over data has shown that α-dystroglycan levels decrease relative to β-dystroglycan

levels. Thus, a person having skill in the art would not be required to resort to undue experimentation to resolve this issue.

Therefore, the claimed invention is predictable and does not require undue experimentation. Sufficient working examples of how to make and use the invention are provided by the specification data, provided by the Campisi Declaration, and post-filing confirmatory data provided, e.g., Exhibits A-E, which confirm the correlation of loss of α-dystroglycan to tumorigenicity. Therefore, Applicants request that the rejection be withdrawn.

B. The declaration of Dr. Judith Campisi is persuasive evidence that in vitro cell line models are accepted as predictive and correlative of tumorigenicity in vivo.

The Office action mailed May 18, 2004, regards the declaration by Dr. Judith Campisi as "an expert opinion," that "does not provide objective evidence as regard[ing whether] the claimed invention is enabled." (Office Action, page 9).

The Office alleges that one of skill in the art would not know how to make or use the invention. Applicants submit that one of skill in the art is informed "how to use the alleged discovery" by the specification. Applicants submit that the Examiner has conceded this is so, by including on page 13 of the rejection, a protocol for a "summer undergraduate student intern" to follow to collect in vivo data. Thus, because the Examiner is able to understand how to make and use the claimed invention, Applicants submit that, indeed, one of skill in the art is also informed how to make and use the claimed invention.

The rejection makes allegations that, it is not clear whether the fragments are circulating in the blood or whether the antibody disclosed could be used to detect the fragments or if they are further degraded. Applicants respond to these allegations in Section C, which addresses objective evidence, because discussion here would cloud the subject of the adequacy of the declaration evidence.

The Office has taken the position that the declaration by one skilled in the art, who is not a named inventor, is not persuasive unless it presented in vivo data to support the claimed invention. Applicants believe the Examiner has not afforded the declaration the weight that it should have been given. Applicants respectfully point out that a purpose of submitting the Declaration by Dr. Judith Campisi was also to provide evidence of the level of skill in the art and to provide evidence that those having skill in the art regard in vitro models as predictive and correlative to in vivo conditions of tumorigenicity. The Examiner has demanded objective evidence and afforded more weight to "data collected by an undergraduate student in terms of enablement than an expert's opinion." (Office action, page 11).

Because Dr. Campisi's declaration did not provide the objective evidence sought by the Office, it appears the declaration was dismissed and not considered on the questions of the level of skill in the art or whether those having skill in the art consider the in vitro models as predictive and correlative to in vivo conditions of tumorigenicity. Therefore, by summarily dismissing Dr. Campisi' declaration and determining that it was unpersuasive, the Examiner has in effect put her opinion above that of the opinion of one agreed upon by both Applicant and the Office as having skill in the art.

The Office puts forth the premise that "there are many differences between cultured cell and their in vivo counterparts" and these cultures "cannot duplicate the in vivo environment in host-tumor and cell-cell interactions." (Office Action, page 12-13).

Applicants recognize that in vitro culture models cannot entirely replace in vivo models. In fact, the inventor, Dr. Mina Bissell, is considered by those in the field to be an early proponent of the use and value of growing cells in three-dimensional (3-D) cultures because these 3-D cultures more closely mimic the in vivo environment. See the news feature, "Cell culture: Biology's new dimension," *Nature* **424**, 870 - 872 (21 August 2003), which discloses that the use of 3-D cultures is becoming a more standard technique. In fact,

because of the value of growing cells in environments that closely mimic in vivo, Applicants performed Examples 3 and 5 in three-dimensional cultures, therefore showing that inhibiting dystroglycan shedding by cancer cells will revert the cells to the normal phenotype.

However, the fact that there are differences in in vitro cell lines and their in vivo counterparts is not dispositive that there is no correlation between what occurs in vitro and in vivo. Neither is the fact that two-dimensional cultures are not entirely reflective of the in vivo environment dispositive in dismissing any data performed in two-dimensional cultures as not predictive or correlative.

Applicants submit in support of patentability, Exhibits F, G, H, I and J for the premise that in vitro models are accepted by those having skill in the art as predictive and correlative of tumorigenicity.

- **Exhibit F**, Schmeichel KL and Bissell MJ, Modeling tissue-specific signaling and organ function in three dimensions, *Journal of Cell Science* 116, 2377-2388 (2003), which is co-authored by one of the inventors, describes the three-dimensional culture in vitro models.

- **Exhibit G,** Voskoglou-Nomikos T, et al., Clinical Predictive Value of the *in Vitro* Cell Line, Human Xenograft, and Mouse Allograft Preclinical Cancer Models, Clinical Cancer Research Vol. 9, 4227-4239, September 15, 2003, which concludes that the "results suggest that under the right framework and when panels are used, the *in vitro* cell line and human xenograft models may be useful in predicting the Phase II clinical trial performance of cancer drugs."

- **Exhibit H,** Abstracts of studies examining the clinical predictive value of preclinical cancer models outside the scope of the NCI screening programs cited within Exhibit G.
    - Bellet R. E., Danna V., Mastrangelo M. J., Berd D. Evaluation of a "nude" mouse-human tumor panel as a predictive secondary screen for cancer chemotherapy agents. *J. Natl. Cancer Inst.* (Bethesda), *63:* 1185-1187, 1979.
    - Bailey M. J., Gazet J-C., Smith I. E., Steel G. G. Chemotherapy of human breast-carcinoma xenografts. *Br. J. Cancer, 42:* 530-536, 1980.

- Inoue K., Fujimote S., Ogawa M. Antitumor efficacy of seventeen anticancer drugs in human breast cancer xenograft (MX-1) transplanted in nude mice. *Cancer Chemother. Pharmacol., 10:* 182-186, 1983.
- Steel G. G., Courtenay V. D., Peckhan M. J. The response to chemotherapy of a variety of human tumor xenografts. *Br. J. Cancer, 47:* 1-13, 1983.
- Taetle R., Rosen F., Abramson I., Venditti J., Howell S. Use of nude mouse xenografts as preclinical drug screens: *in vivo* activity of established chemotherapeutic agents against melanoma and ovarian carcinoma xenografts. *Cancer Treat. Rep., 71:* 297-304, 1987.
- Mattern J., Bak M., Hahn E. W., Volm M. Human tumor xenografts as models for drug testing. *Cancer Metastasis Rev., 7:* 263-284, 1988.
- Boven E., Winograd B., Fodstad O., Lobbezzoo M. W., Pinedo H. M. Preclinical Phase II studies in human tumor lines: a European multicenter study. *Eur. J. Cancer, 24:* 567-573, 1988.
- Boven E., Winograd B., Berger D. P., Dumant M. P., Braakhuis B. J. M., Fodstad O., Langdon S., Fiebig H. H. Phase II preclinical drug screening in human tumor xenografts: a first European multicenter collaborative study. *Cancer Res., 52:* 5940-5947, 1992.
- Langdon S., Hendriks H. R., Braakhuis B. J. M., Pratesi G., Berger D. P., Fodstad O., Fiebig H., Boven E. Preclinical Phase II studies in human tumor xenografts: a European multicenter follow-up study. *Ann. Oncol., 5:* 415-422, 1994.

- **Exhibit I,** L. A. Kunz-Schughart, J. P. Freyer, F. Hofstaedter, and R. Ebner, The Use of 3-D Cultures for High-Throughput Screening: The Multicellular Spheroid Model, *J Biomol Screen*, June 1, 2004; 9(4): 273 – 285, which explore the use of 3-D cultures for high-throughput screening.

- **Exhibit J,** Holbeck SL, Update on NCI in vitro drug screen utilities, *Eur. J. Cancer* 40 (2004) 785-793.

Applicants submit that these Exhibits, together with Dr. Judith Campisi's declaration, show that "a number of *in vitro* cell culture models are generally recognized in the art as correlating to *in vivo* conditions of tumorigenicity or tumorigenic potential." (Declaration of Dr. J. Campisi, paragraph 5). Moreover, the broad use in the art of in vitro cell lines is further evidence of the acceptance of in vitro models as generally predictive of in vivo conditions. One need look no further than the thousands of cell lines available through the American Type Culture Collection (ATCC) and the 60-cell line panel utilized by the National Cancer Institute (NCI) which is discussed in Exhibit J.

Therefore, Applicants respectfully request that the rejection be withdrawn based on the evidence provided in the Declaration of Dr. Judith Campisi, of record, and Exhibits F-J, submitted herewith. Based on the evidence provided, the Office is urged to conclude that one having skill in the art would consider in vitro cell line models to be both predictive and correlative of tumorigenicity in vivo.

C. The claims do not require objective evidence for enablement.

The Examiner has maintained the position that an in vivo working example is required. (Office action, page 11). However, the Office, the MPEP and case law do not require *in vivo* examples for enablement. Applicants refer to MPEP 2164.04:

> [The] examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure.
>
> As stated by the court, [in *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971)] "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement.

In re Brana, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995) and MPEP 2164.02 support Applicant's position that an in vitro animal model example in the specification constitutes a working example since that example correlates with a disclosed or claimed method invention. Again according to MPEP 2164.02,

> The issue of "correlation" is related to the issue of the presence or absence of working examples. "Correlation" as used herein refers to the relationship between in vitro or in vivo animal model assays and a disclosed or a claimed method of use. **An in vitro or in vivo animal model example in the specification, in effect, constitutes a "working example" if that example "correlates" with a disclosed or claimed method invention.**
>
> Since the initial burden is on the examiner to give reasons for the lack of enablement, the examiner must also give reasons for a conclusion of lack of

> correlation for an in vitro or in vivo animal model example. **A rigorous or an invariable exact correlation is not required,** as stated in Cross v. Iizuka, 753 F.2d 1040, 1050, 224 USPQ 739, 747 (Fed. Cir. 1985). (emphasis in bold added).

Since clearly the law and the MPEP support Applicant's position that an in vitro animal model example in the specification constitutes a working example. Perhaps the rejection the Office is trying to make is that of utility in conjunction with enablement. Under MPEP 2164.07, the Examiner has the initial burden to show that one of ordinary skill in the art would reasonably doubt the asserted utility. In fact, it appears that this is the rejection so intended as seen by the allegation that "it is not clear whether the 120-130 kD or the 60kD fragment is circulating in the blood of the fragments are further degraded." (Office Action, page 10). Applicants assert that the Examiner has not met the burden to show that one of ordinary skill in the art would reasonably doubt the asserted utility based upon the evidence provided by Applicants.

Furthermore, Applicants "do[] not have to provide evidence sufficient to establish an asserted utility is true "beyond reasonable doubt." (*In re Irons*, 340 F.2d 974, 978, 144 USPQ 351, 354 (CCPA 1965). Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true." (MPEP 2164.07(I)(B)).

The rejection has appeared to take the improper standard of "beyond reasonable doubt" as seen by the last paragraph of pages 14-15, wherein the rejection asks:

> If a...fragment of α-dystroglycan is detected from the blood sample of a subject...what does it not mean? Does that subject have a higher tumorigenic potential to develop leukemia, or breast cancer, or something else? How about a strong signal in an immunoblot vs. a weak signal? When [would] one expect [a] false positive vs. false negative? What is the normal control value?

It appears the rejection is demanding proof that is beyond what can be obtained in clinical trials, which is a greater requirement than the Office's standard of "more likely than not."

Applicants briefly respond to these questions although Applicants have difficulty understanding this quoted comment. According to the specification, shedding of α-dystroglycan is indicative of tumorigenicity, which is not expressly limited to breast cancer. This is confirmed by Exhibits B and E which report that reduced or loss of α-dystroglycan correlates to breast, prostate and oral cancers. As for questions involving signal strength, false positives/negatives, normal control value, Applicants respectfully submit that one having skill in the art would know how to make and use the invention so as to minimize false positives/negatives, establish proper controls, and understand and establish calibration curves that correlate signal strength to tumorigenic potential. In support, Applicants point to paragraph 4 of Dr. Campisi's declaration, where she states:

> Establishing a calibration curve and correlating this to tumorigenic potential would be a routine procedure that would be carried out in any given laboratory for a specific set of reagents and reaction conditions, according to basic scientific principles. Although some experimentation would be necessary to achieve the results described referred to in this paragraph, such experimentation would be straightforward, given the basic teachings of this patent application and the findings set forth in the patent application.

Applicants assert that one of ordinary skill in the art would not (a) reasonably doubt the asserted utility that α-dystroglycan can be detected in blood serum to indicate potential tumorigenicity or (b) doubt that one of skill could detect α-dystroglycan in the blood. It is widely recognized that the detection of proteins in the serum can serve as a diagnostic measure of tissue-specific diseases. Applicants submit Exhibits K, L and M, herewith, to describe the usefulness and value of of studying serum proteins as diagnostic markers.

- **Exhibit K,** Stevens EV, Liotta LA, Kohn EC, Proteomic analysis for early detection of ovarian cancer: a realistic approach?, *Int J Gynecol Cancer*. 2003 Nov-Dec;13 Suppl 2:133-9.

- **Exhibit L,** Vlahou A, Laronga C, Wilson L, Gregory B, Fournier K, McGaughey D, Perry RR, Wright GL Jr, Semmes OJ, "A novel approach toward development of a rapid blood test for breast cancer," *Clin Breast Cancer.* 2003 Aug;4(3):203-9.

- **Exhibit M,** Bidart JM, Thuillier F, Augereau C, Chalas J, Daver A, Jacob N, Labrousse F, Voitot H, Kinetics of serum tumor marker concentrations and usefulness in clinical monitoring, *Clin Chem.* 1999 Oct;45(10):1695-707.

As stated in Exhibit K, Stevens EV et al., "[s]erum is a key source of putative protein biomarkers, and, by its nature, can reflect organ-confined events."

Moreover, many proteins that are shed from cancer cells have been succesfully detected in the serum of cancer patients. See references provided as Exhibits N, O, P and Q, submitted herewith, which describe proteins shed from cancer cells, as examples of such serum detection.

- **Exhibit N,** Aoki T, Yonezawa K, Ohuchi E, Fujimoto N, Iwata K, Shimada T, Shiomi T, Okada Y, Seiki M, Two-step sandwich enzyme immunoassay using monoclonal antibodies for detection of soluble and membrane-associated human membrane type 1-matrix metalloproteinase, *J Immunoassay Immunochem.* 2002;23(1):49-68.

- **Exhibit O,** Taylor DD, Gercel-Taylor C, Gall SA, Expression and shedding of CD44 variant isoforms in patients with gynecologic malignancies, *J Soc Gynecol Investig.* 1996 Sep-Oct;3(5):289-94.

- **Exhibit P,** Tsujisaki M, Imai K, Hirata H, Hanzawa Y, Masuya J, Nakano T, Sugiyama T, Matsui M, Hinoda Y, Yachi A, Detection of circulating intercellular adhesion molecule-1 antigen in malignant diseases., *Clin Exp Immunol.* 1991 Jul;85(1):3-8.

- **Exhibit Q,** Vangsted AJ, Clausen H, Kjeldsen TB, White T, Sweeney B, Hakomori S, Drivsholm L, Zeuthen J, Immunochemical detection of a small cell lung cancer-associated ganglioside (FucGM1) antigen in serum, *Cancer Res.* 1991 Jun 1;51(11):2879-84.

- Wirth et al., Value of prostate-specific antigen as a tumor marker, *Eur Urol* 1993; 24 Suppl 2:6-12, of record.

Thus, as shown in the Exhibits N through Q and Wirth et al., of record, the detection of shed proteins in serum can be done, and has been done repeatedly.

The detection of these proteins depends most importantly on the availability of good antibodies. In some embodiments, a dual antibody approach can increase sensitivity. With the application of the dual antibody approach, proteins have been detected in serum that were previously below detection levels. Arguably, any shed protein can be detected in the serum with the application of this highly sensitive approach. Furthermore, it is known in the art that objective measures of shed protein levels can be correlated with disease whether those levels are high, low or undetectable.

The inventors have detected shedding of α-dystroglycan in cultured in carcinoma cells. Currently, α-dystroglycan detection is limited to only two antibodies, both of which are low-affinity carbohydrate-dependent antibodies that bind a similar epitope (Ervasti JM, Ohlendieck K, Kahl SD, Gaver MG, Campbell KP: Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. *Nature* 1990, 345:315-319, not attached). However, altered glycosylation of dystroglcyan occuring in carcinoma cells may render this molecule undetectable by these same antibodies (see Singh et al., Proteolytic enzymes and altered glycosylation modulate dystroglycan function in carcinoma cells, *Cancer Res.* 2004 Sep 1;64(17):6152-9, not attached). Therefore new, more specific antibodies may be required for successful detection of dystroglycan in the serum of human cancer patients.

The Office has conceded that the methods of making monoclonal antibodies and of screening for proper antibodies are well known to persons skilled in the art, therefore, it should be a matter of routine experimentation to obtain specific antibodies to the α-dystroglycan fragments shed into bodily fluids such as blood or serum. (*Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986).

Given the state of the art, it is clear that an in vivo working example is not required. As is demonstrated in the art, any doubt by the Office as to sufficiency of the working examples is unfounded. For confirmatory data, Applicants again point the Office to

Sgambato et al., "Dystroglycan Expression is frequently reduced in human breast and colon cancers and is associated with tumor progression," *Am J Pathol.* March 2003, vol.162, no.3, 849-860, Exhibit E. Sgambato et al. investigated expression of both α-dystroglycan and β-dystroglycan in both human cancer cell lines and in primary colon and breast cancers. The results of Sgambato et al. mirror the results found earlier by Applicant. Sgambato et al. go on to state that, "We found that α-DG expression is frequently lost in breast cancer cells and observed a progressive loss of its expression with advancing tumor stage."

Therefore, the weight of the existing literature indicates that, with the mere creation of better antibodies in combination with, *e.g.*, known dual-antibody detection methods, shed portions of α-dystroglycan are detected in serum. Thus, Applicants submit that they have provided evidence sufficient to establish an asserted utility and enablement. When considered as a whole, this evidence leads a person of ordinary skill in the art to conclude that the asserted utility of detection of α-dystroglycan in blood serum is true under the more likely than not standard. Applicants also submit that the evidence provided is sufficient to show one having skill in the art how to make and use the claimed invention. Applicants, therefore, request that the rejection based on lack of enablement (which actually appears to be a rejection based on lack of utility) be withdrawn.

In conclusion, Applicants have submitted substantial evidence and argument showing that the claimed invention is enabled. The claimed invention is predictable and would not require undue experimentation to practice. Based on case law, the MPEP, the declaration of one of skill in the art, the level of skill in the art as well as the accepted practices of those in the art, the disclosure in the specification and the confirmation and correlation by others, one of skill in the art would not require objective evidence in an in vivo animal model in order to make and use the invention. Applicants respectfully request that the requirement of providing objective evidence be withdrawn and the instant claims be allowed.

2. Claim 8 is not indefinite under 35 U.S.C. § 112 second paragraph.

Claim 8 is currently amended. Applicants point to support for the amendment at pages 13-16. Applicants submit that amended claims are definite and the rejection should be withdrawn.

CONCLUSION

For the reasons set forth above, Applicants respectfully request that the accompanying amendments to the claims be entered and considered for this case. Applicants respectfully urge the Examiner to withdraw all rejections in view of the arguments presented.

Applicants have requested a Three-Month Extension of time from August 18, 2004 to November 18, 2004. A petition for an extension of time is included herewith in duplicate. Please charge fees in accordance with the enclosed fee calculation sheet. Please charge any necessary and additional fees that may be due to Deposit Account No. 120690.

Should the Examiner believe that a telephone interview would aid in the prosecution of this application, Applicants encourage the Examiner to call the undersigned at (510) 495-2456.

Respectfully submitted,
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